



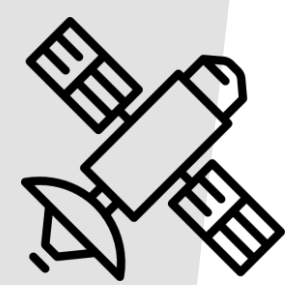
Introduction

While sponges are generally known to host a wide range of microbial associates, the composition and specificity of the microbial communities in carnivorous sponges are poorly understood. We used 16S-rRNA gene data and supporting experiments to examine and compare the bacterial communities associated with distinct anatomical regions of two carnivorous sponge species from the Canadian arctic.



Study goals

- Investigate similarities and differences in the bacterial communities associated with *Chondrocladia grandis* and *Cladorhiza oxeata* (Fig. 1), and determine which factors best explain assemblage composition (Fig 2, 3 & 4).
- Identify bacterial biomarkers for each anatomical region (Fig. 5) of these carnivorous sponges (i.e. taxa that are consistently and significantly enriched).
- Characterize the oligotype stability of common and biomarker taxa across host specimens from various geographical regions and depths (Fig 6).



Sample collection

Chondrocladia grandis (N=7) and *Cladorhiza oxeata* (N=2) were sampled from Baffin Bay and the Gulf of Maine (Fig. 1).

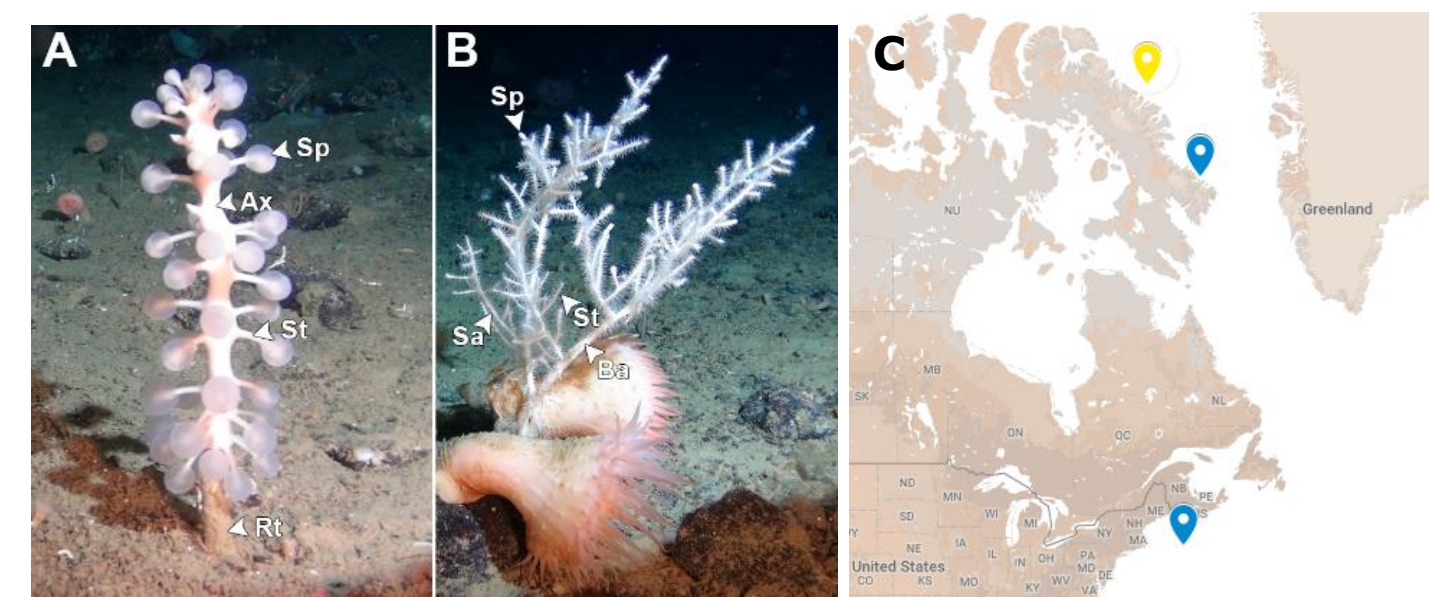
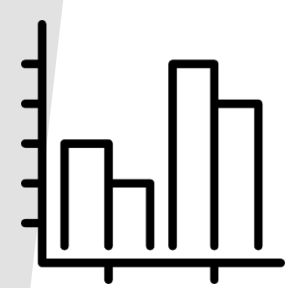


Fig. 1: *C. grandis* (A) and *C. oxeata* (B) as observed *in situ*. (sp) sphere, (ax) axis, (st) stem, (rt) root. (ba): base axis, (sa): secondary axis (C) Collection sites, blue: *C. grandis*, Yellow: *C. oxeata*



Microbiome richness

Large differences in bacterial diversity and evenness were observed between the two sponge species (Fig. 2). Microbial communities are not evenly distributed amongst anatomical regions. Specifically, within *C. grandis* the root and root-tip samples displayed the most diverse communities, while axis, sphere and stem were markedly less diverse.

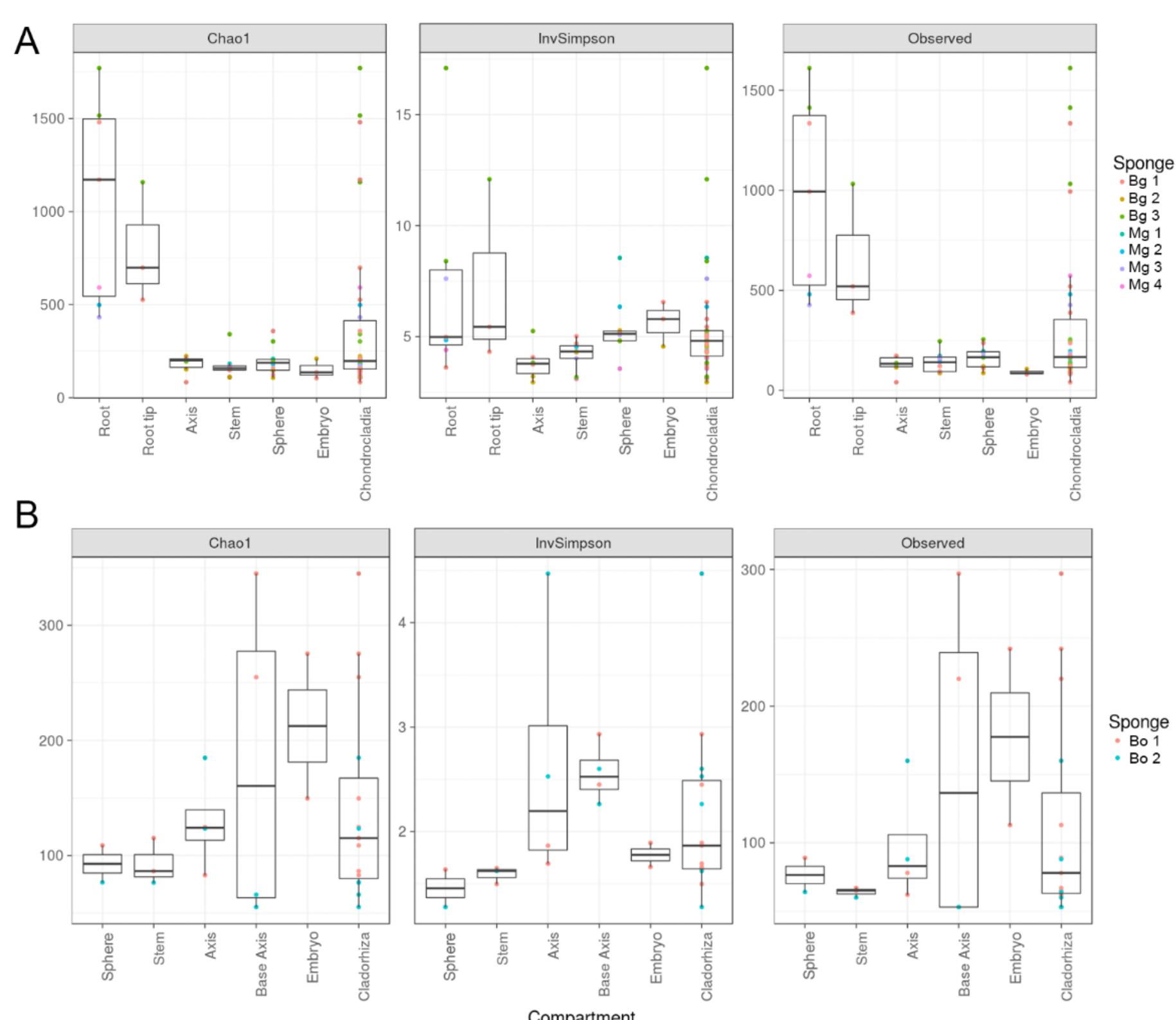


Fig. 2: Alpha diversity metrics per sponge and sponge compartment (A) *C. grandis* (B) *C. oxeata*. B: Baffin Bay; M: Gulf of Maine

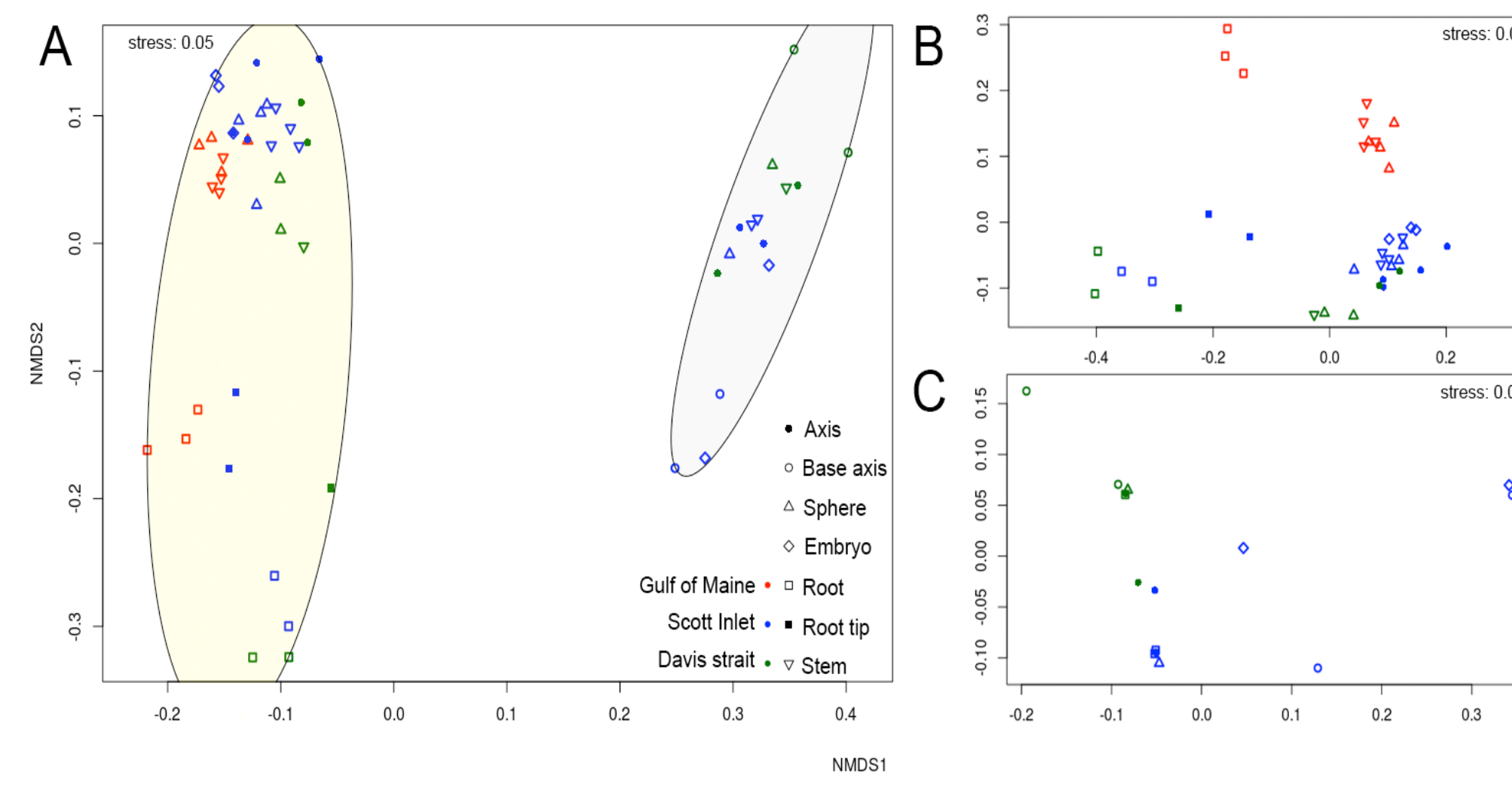


Fig. 3: Ordination analysis (NMDS) bacterial. Displayed are (A) Combined *Chondrocladia grandis* (left cluster) and *Cladorhiza oxeata* (right cluster) samples from Davis Strait (green), Gulf of Maine (red) and Scott Inlet (blue) (B) *Chondrocladia grandis* samples, and (C) *Cladorhiza oxeata* samples. Symbols indicate anatomical regions (see Fig. 1).

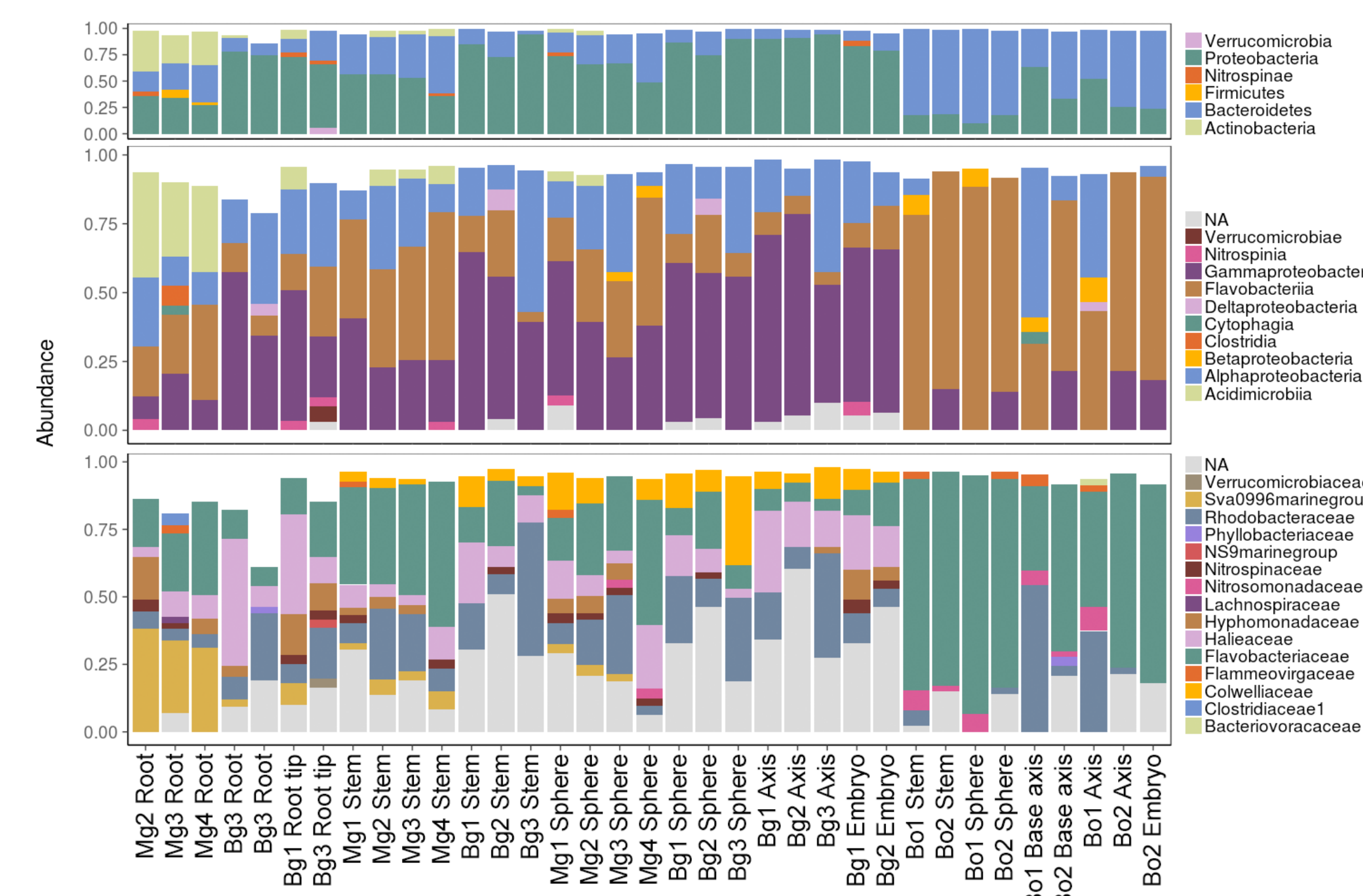


Fig 4: Most common bacterial taxa found in anatomical regions of *C. grandis* and *C. oxeata*. X-axis represents anatomical regions (grouped per sponge individual) while color-coded segments in each bar indicate taxa with a relative abundance higher than 2%, using count data agglomerated at Phylum (top panel), Class (middle panel) and Family (bottom panel) levels. Sponge individuals are represented by different numbers and are coded by broader geographic region (B: Baffin Bay; M: Gulf of Maine) and species (g: *C. grandis*; o: *C. oxeata*).

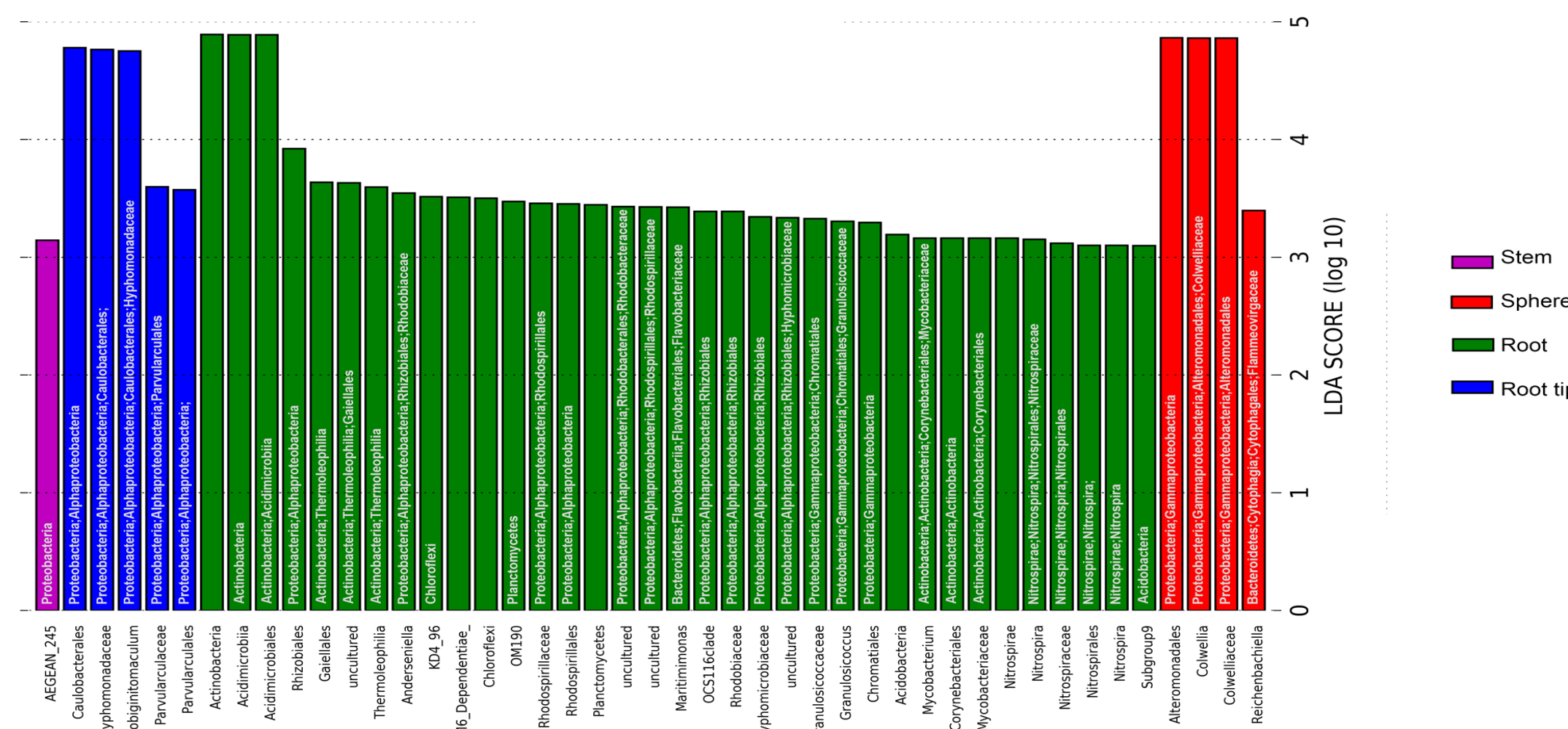


Figure 5: Differentially abundant bacterial genera (biomarkers) in *Chondrocladia grandis*. Each bar indicates the LDA score for a specific biomarker that is significantly ($p < 0.01$) more abundant within the respective anatomical region (indicated by bar color). The most specific taxonomy is given on the Y axis, while additional taxonomic information is displayed within each bar (if available).

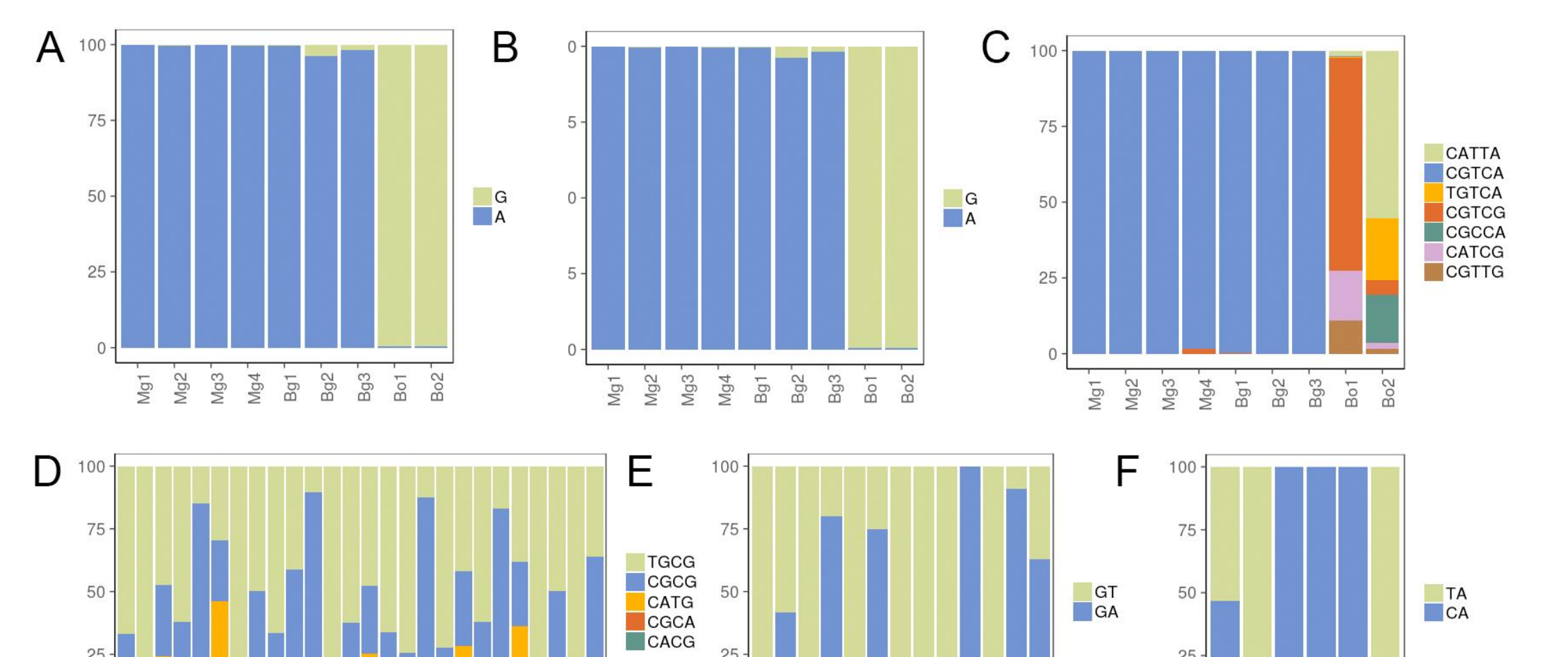
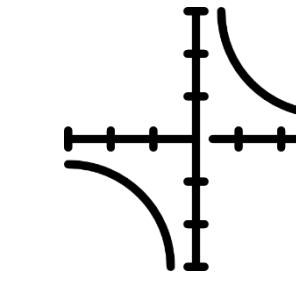
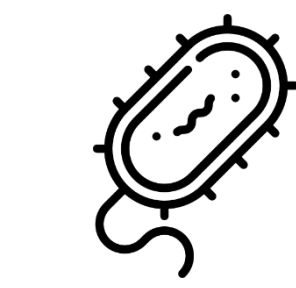


Figure 6: Oligotype results of both common and biomarker. Bars represent anatomical regions per sponge, while stacked bars show the relative abundance of each oligotype within the common genera (A) *Tenacibaculum*, (B) *Candidatus Branchiomonas Fulvivirga* as well as the *C. grandis* specific biomarkers (D) *Colwellia*, (E) *Reichenbachella* and (F) *Maritimimonas*. Comparison of common genera oligotypes is presented grouped on sponge individual, while biomarker oligotypes are displayed per anatomical region.



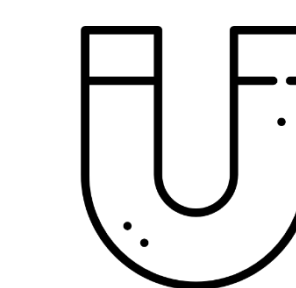
Comparison of microbiomes

Systematic differences between the bacterial communities of the two sponge species were observed (Fig. 3). In *C. grandis*, a significant difference in bacterial composition among anatomical regions was also seen, with unique assemblages of bacteria within the root and root tip samples setting these regions apart from the remainder of the sponge body. Geographical location and host individual also influenced the composition of the microbiome (Figure 3B), albeit with a smaller effect size. Clustering of *C. oxeata* samples within the ordination analysis (Fig. 3C) could not be explained by available variables within our dataset and appeared more diffuse.



Bacterial community composition

Bacterial communities (Fig. 4) in both sponge species included a high abundance of Flavobacteriia, γ -proteobacteria and α -proteobacteria). At family level, differences between *C. grandis* and *C. oxeata* were observed in terms of bacterial abundance and richness. In *C. oxeata*, the only ubiquitous family was the Flavobacteriaceae, whose members represented the majority of the community. *C. grandis* showed a larger diversity, with abundance values distributed amongst the most common families including the Flavobacteriaceae, Rhodobacteraceae, Halieaceae, Colwelliaceae and Hyphomonadaceae.



Biomarker detection

While no biomarker taxa were found for *Cladorhiza oxeata*, the association between specific bacterial taxa and anatomical regions does occur within Arctic *C. grandis* (Fig. 5), in agreement with what previously observed (Verhoeven et al., 2017). These associations may constitute symbiotic relationships, although further functional studies are needed to fully confirm this hypothesis.



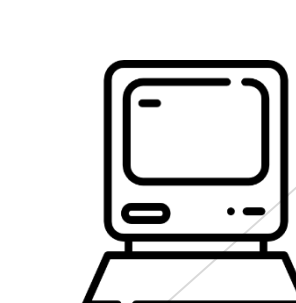
Oligotype specificity

Oligotyping of genera common to both sponge species (*Tenacibaculum*, *Candidatus Branchiomonas* and *Fulvivirga*) as well as biomarker genera, showed a host species-specific pattern of bacterial oligotypes, consistent throughout the entire sponge body. Within *C. grandis* this specificity remains unchanged even across a wide geographical distance (Fig 6).



Discussion

The two investigated sponge species hosted distinct bacterial communities, with taxonomic diversity being greater in *C. grandis*. Some bacterial taxa (including particular oligotypes) were consistently recovered in multiple host individuals from geographically distant sites, suggesting specificity. Within *C. grandis*, several bacterial taxa were enriched in particular anatomical regions, suggesting functional roles in carnivorous sponge metabolism or other biological processes. The observed differences in bacterial community composition and diversity between *C. grandis* and *C. oxeata* may reflect differences in trophic adaptability, specialization and overall reliance on associated bacteria.



References

- Verhoeven, J.T.P., Kavanagh, A.N., Dufour, S.C., 2017. Microbiome analysis shows enrichment for specific bacteria in separate anatomical regions of the deep-sea carnivorous sponge *Chondrocladia grandis*. *FEMS Microbiol. Ecol*
- Verhoeven, J.T.P. and Dufour, S.C., 2017. Microbiomes of the Arctic carnivorous sponges *Chondrocladia grandis* and *Cladorhiza oxeata* suggest a specific, but differential involvement of bacterial associates. *Arctic Science*